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THE EFFECT OF THE HIGH TEMPERATURE OF THE ENVIRONMENT ON VARIOUS PHYSIOLOGICAL AND BIOCHEMICAL REACTIONS OF
AN ORGANISM

A. Kh. Babayeva

Overheating of the human and animal organism evokes a number of complex pathological changes in vital processes. Interesting studies have been carried out in the past decades which showed that on overheating there occur disorders in the functions of the central nervous system, endocrine glands, thermoregulation processes, water and salt balance, basal metabolism, protein metabolism, and the intensity of oxidation-reduction processes in tissues of an organism.

In spite of the relatively large number of investigations, no actual preventive measures against overheating have been suggested so far. This is explained by the fact that the problems of the prevention and therapy of overheating can be solved only by knowing the profound and fine mechanisms of adaptation and the patterns in the pathogenesis of overheating. But these mechanisms and patterns have not yet been revealed.

This journal publishes a selection of articles which are united by a common comprehensive problem. The article summarized results of

investigations which were obtained by six authors who made a comprehensive study of pathological deviations ensuing upon overheating in the same animals.

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VARIATION IN THE SODIUM AND POTASSIUM CATION CONCENTRATION IN RABBIT
BLOOD PLASMA ON OVERHEATING*

A. Kh. Babayeva

An investigation of disorders in the water volumes and electrolytic composition of the aqueous environments of animals is of particular interest during overheating. The requirements of thermoregulation cause a shift of water and salt in an animal organism, in particular, a redistribution of the cations and anions between the intra- and extracellular aqueous environments. A change in the electrolytic composition of the intracellular environment of an organism is reflected in biochemical metabolism [2, 7, 8]. Variations in the sodium and potassium concentrations play an important role in protein, carbohydrate and energy metabolisms. The sodium ion plays a particular role in the regulation of water translocation in the animal organism.

The purpose of our investigation was to trace the changes in the sodium and potassium concentrations on overheating.

* The work on the flame photometer was carried out in the chemical laboratory of the Central Thematic Comprehensive Expedition. We wish to thank N. I. Breunov for his help.

Procedure and results of the investigations. The experiments were carried out on 36 rabbits of about the same age weighing 1.5-2 kg. The diet in the experimental animals was mixed and during all experiments was not changed. Overheating of the animals was carried out under artificial (a special heat chamber) and under natural conditions. The heat chamber was a rectangular box 100 x 90 x 70 cm with double walls with felt between them. Inside the chamber were thirty 60-watt bulbs. We placed in the chamber a metal cage whose walls had numerous small openings for ventilation. Two peep-holes were on the front wall of the chamber. The temperature was observed by a thermometer in the chamber; we regulated it by means of a laboratory autotransformer, increasing or decreasing the heat of the electric bulb.

Four series of experiments were set up: a) a single overheating in the heat chamber for one hour at 45°; b) chronic exposure to heat (36°) in the chamber for 14 days of one hour daily; c) acute overheating of the rabbits that were preliminarily subjected to chronic heat exposure; d) overheating under natural conditions (in a solarium) for one hour at 43-45°.

Determinations of all indexes in the urine collected during the day were performed in addition to determinations of the sodium and potassium in blood plasma of animals that underwent the effect of heat under natural conditions. The body weight (before and after overheating) was determined, the rectal temperature was measured (by an electrothermometer), and the behavior of the animals was observed. The rabbits were killed by decapitation.

We determined the sodium and potassium in the blood plasma and in the daily amount of urine before and after overheating. To determine the sodium and potassium we used flame photometry, which in recent years has found ever greater application in experimental medico-biological

laboratories and in the clinic. Analyses are carried out with greater accuracy and incomparably more rapidly by means of flame photometry than by weight or volume methods.

The standards for determining the sodium and potassium concentrations in blood plasma and urine were made by the method suggested by M. G. Zaks and G. S. Chudnovskiy [5, 6]. The materials for the investigation were prepared in the following manner. Heparinized plasma in an amount of 2 ml was introduced into a measuring flask (100 ml) and distilled water added to the mark. After mixing, the solution was ready for the investigation. The urine was diluted on the basis of the calculation of 1 ml per 100 ml of water. The photometric data of the standard solution are shown in Fig. 1.

The data in Table 1 show that under the effects of chronic overheating there is an increase in the sodium level of the blood plasma. In rabbits that were subjected to daily overheating in the hot chamber for 14 days, the blood plasma sodium level increased by 5%. An even greater increase (by 15%) was observed in the experiment set up in June 1961, in which the rabbits were for a long time in a vivarium where the daily air temperature was 36.8-37° and a period of natural adaptation took place.

A decrease of blood plasma sodium was observed in all animals upon acute overheating (45°) in the heat chamber or under natural conditions. The sodium concentration dropped by 7.3% on acute overheating in the control rabbits and the drop was expressed to a lesser degree, by 4.8% in the rabbits that were heat-trained in the chamber. In rabbits adapted to heat under natural conditions the decrease was more appreciable, 16.1%. It is necessary to note that under the effect of acute overheating the blood plasma sodium level dropped to the norm in rabbits which had hypernatremia as a result of adaptation. The changes

we found in kaliemia were weakly expressed and unreliable.

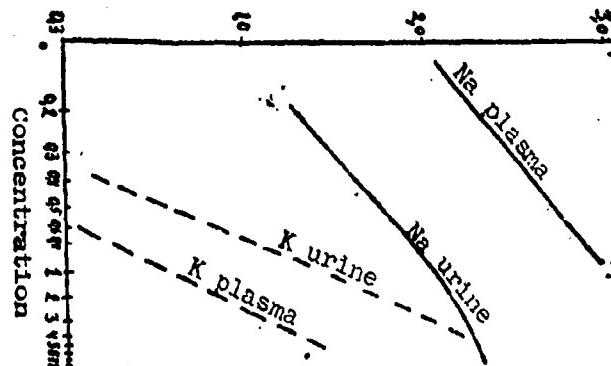


Fig. 1. Photometric graphs of standard solutions.

TABLE 1

Variation in the sodium and potassium concentrations in the blood plasma of rabbits subjected to overheating in a hot chamber (mg%)

Central group		Acute overheating		Adapted		Acute overheating of adapted	
Na	K	Na	K	Na	K	Na	K
340	--	290	20,0	350	21,0	290	--
315	17,0	320	--	325	--	350	20,0
340	18,5	290	19,0	325	18,8	335	18,8
305	21,5	305	18,8	326	18,8	338	--
305	22,0	300	18,5	350	21,0	339	17,0
330	19,0	290	18,8	330	18,8	340	20,0
average(M)	20,2	290,1	18,9	338,0	19,7	322,0	18,9

The variation of the sodium concentration in the blood plasma of rabbits subjected to overheating under natural conditions is presented in the following form:

Adapted Acute over-
 heating in
 solarium

360	285
320	290
355	300
360	330
340	325
330	325
335	—
—	—
365	340
375	305
330	340
—	345
average (M)	379,0
	318,0

Therefore, the results of our investigations showed that the sodium level in blood plasma increases under the effect of chronic overheating. These shifts took place in rabbits adapted to heat both under natural conditions and in a hot chamber. The increase of sodium in the blood plasma of the experimental animals is apparently an expression of adaptation. The blood plasma sodium level of animals decrease under the effect of acute overheating. This decrease is more expressed on acute overheating under natural conditions.

The variation of the sodium concentration in the blood plasma can be explained: a) by the increase of the blood plasma volume, i.e. its liquification due to the uptake of water; b) by the rapid elimination of sodium through the kidneys; c) by the passage of sodium from the blood plasma into the intracellular space (erythrocytes and tissues), which can be caused by the development of hyperventilation alkalosis in which the need to retain electroneutrality leads to a decrease of the cation concentration.

We found that on overheating, the elimination of sodium and potassium in the urine as well as the magnitude of diuresis decrease. A considerable reduction in the elimination of these cations was noted in rabbits that underwent acute overheating under natural conditions. Consequently, we cannot speak about a rapid elimination of cations

through the kidneys. We think that in our investigations the change in the cation content was associated with a redistribution of fluid in the rabbits. This is in accord with the data in the literature [3, 4]. It is also known that a state of hyperventilation alkalosis ensues under the effect of overheating [1]. Therefore, it is possible that the change in sodium is also partially the consequence of gaseous alkalosis arising in experimental animals under the effect of acute overheating.

Conclusion

1. The sodium concentration in blood plasma increases upon the chronic effect of overheating.
2. A decrease of the sodium level in blood plasma occurs under the effect of acute overheating of control and adapted animals. This decrease is more expressed on overheating under natural conditions.

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VARIATION OF THE BLOOD SERUM PROTEIN COMPOSITION UPON OVERHEATING

N. N. Stefanovskaya

The amount of total protein and the ratio of the protein fractions of blood serum are relatively constant. A study of blood proteins upon overheating is of considerable interest under hot climatic conditions. In a number of investigations definite regularities were noted between the degree of overheating and shifts in the protein composition of blood serum. Therefore, a change in serum proteins is proposed as a test when studying the effect of high temperatures on an organism [3].

Data are available which indicate that at an elevated temperature of the environment the breakdown of tissue proteins is accelerated, the excretory function of the kidneys is impaired, nitrogen metabolism is enhanced, and the protein requirement of an organism increases [5, 11].

The amount of residual nitrogen and urea in the blood of the hepatic vein and tissues of the liver increases in rabbits under acute hyperthermia [8].

A change in the fractional composition of blood serum protein depends on the dose of solar radiation. Sunbaths of 5-35 calories do not affect the ratio of blood serum protein fractions in man. An increase in the dose to 45-60 calories is accompanied by hyperglobulinemia [2].

At a daily 3-hour effect of a temperature of 40°, wave-like (with a period of one month) oscillations of the total protein in blood serum were noted in rabbits. By the end of the fifth month of overheating the globulin level increases in them [7].

Overheating of dogs leads to a decrease in the albumin concentration and an increase of globulins [4]. Other authors, when overheating dogs and guinea pigs for 1-2 hours at 40-42°, observed an increase in the total blood serum protein, albumin, an increase in the albumin/globulin quotient, and a decrease of globulins and fibrogen [14].

The data of Z. N. Lebedeva [12] concerning the increase in the level of free amino acids in rabbit blood upon overheating indicate a disorder of the processes of protein synthesis and resynthesis. Experiments with labeled glycine also confirm the hypothesis of a disorder in protein synthesis upon overheating [15].

In the present report we will cite data on the content of total protein and ratio of the protein fractions in blood serum and in an extract of soluble liver proteins upon overheating experimental animals in a heat chamber and in a solarium. The experiments were carried out on 12 white rats and 32 rabbits.

Methods of investigation. The amount of total protein in the blood serum and extract of soluble liver proteins was determined by the gravimetric method [2]. To separate the blood serum proteins we used the method of paper electrophoresis. The method for obtaining the soluble liver proteins is cited in the works of S. Ya. Kaplinskiy et al. [9, 10]. For this purpose the rat liver was washed clear of blood and triturated with a physiological solution (1:1). The homogenate was frozen and left in the evaporator of a cooler for 24 hours. The homogenate was thawed, small portions of ether was added and centrifuged. We transferred the liquid above the precipitate to a test tube, placed it

for ten minutes in a water bath at 37°, centrifuged, and a yellowish transparent extract of soluble liver proteins was obtained. Electrophoresis of the extract was carried out in a borate buffer with pH 8.6 for six hours at a voltage of 300 v. At the same time we set up for electrophoresis the blood serum of a given animal in order to compare the fractions obtained.

We must point out that the amount of liver protein fractions obtained by different authors is dissimilar. The number of fractions of soluble liver proteins varies from two to nine [10,13]. In our experiments we obtained four fractions of soluble liver proteins which correspond to serum proteins with respect to the velocity of moving on the electrophoregram. These fractions were designated by the letters A, B, C, D. The data obtained were treated by the method of statistical variations. We calculated the arithmetical mean (M), the mean error (m), and the criterion of the reliability of the difference in results (P). Data with $P < 0.05$, i.e. when the probability of the difference was 95%, were considered reliable.

Results of the investigations. The first series of experiments was carried out on white rats (males) weighing 185-234 g, overheated in a hot chamber for 30 minutes at 40°. The group of rats not subjected to overheating was the control. In the rats we determined the total protein of the blood serum and its fractions, the total protein of the extract of soluble liver proteins, and we fractioned the soluble liver proteins (Table 1). Upon a single overheating of the rats the amount of protein in the abstract of the soluble liver proteins decreased in fractions B, C, D, by 1.3 g % (20%). The amount of total blood serum protein decreased by 0.8 g % (10.8%), albumin by 0.5 g % (19.9%); the albumin/globulin quotient decreased to 0.4%.

The second series of experiments was carried out on rabbits who were subjected for one hour a day for 20 days to a temperature of 36° in a heat chamber. We determined the content of total blood serum protein and the ratio of its fractions in the rabbits. The results were compared with the data obtained in the control group of animals (Table 2). Changes in the concentration of blood serum proteins were not observed in the rabbits adapted to a temperature of 36°; the albumin/globulin coefficient did not vary ($P < 0.5$).

The third series of experiments included rabbits adapted to a temperature of 36° for 14 days and on the 15th day they were overheated for one hour at 45° in a heat chamber. In the rabbits we noted a decrease in the amount of total serum protein at the expense of globulin (by 0.2%); the albumin/globulin quotient increased to 1.36.

The next experiments were carried out on four rabbits subjected to one hour overheating at 45° in a heat chamber. Because of the small number of experimental animals the data obtained in these experiments were not processed by the statistical variation method. The content of total protein in the blood serum in this group of rabbits was 6.2 g %, albumin 3.5 g % (56.5%), α -globulin 0.6 g % (10.1%), β -globulin 0.9 g % (14.1%), γ -globulin 1.2 g % (19.3%). A decrease in the amount of serum protein by 10.1% was observed in comparison with the control. The content of the globulin fractions noticeably dropped, the protein coefficient increased to 1.3. The shifts in the protein composition of the blood of rabbits not adapted to high temperature were expressed more distinctly.

Further experiments were carried out in June 1961. We investigated the variation of serum proteins under the effect of solar irradiation. The blood samples were taken from rabbits before and after an hour of overheating; in a solarium at 45-47°; two rabbits died upon overheating.

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TABLE 1
Total protein, protein fractions in blood serum and soluble liver proteins of white rats in norm and on overheating

Treatment group and series	N. num.	Soluble liver proteins		Blood Serum	Soluble liver proteins
		Control series	Overheating series		
A-B	2	26 ± 0.14	0.9 ± 0.10	3.1 ± 0.04	3.8 ± 0.11
C-D	2	1.5 ± 0.07	1.7 ± 0.14	1.4 ± 0.22	1.5 ± 0.09
E-F	2	1.5 ± 0.25	1.9 ± 0.25	1.3 ± 0.25	P < 0.05
G-H	2	1.9 ± 0.21	1.9 ± 0.21	1.8 ± 0.24	P > 0.5
I-J	2	0.54 ± 0.04	—	6.6 ± 0.21	1.3 ± 0.08
K-L	2	35.0 ± 1.6	1.2 ± 1.41	0.45 ± 0.02	5.2 ± 0.19
M-N	2	39.5 ± 1.5	27.3 ± 2.44	—	P < 0.02
O-P	2	19.9 ± 0.53	33.1 ± 2.61	31.8 ± 0.86	16.5 ± 1.54
Q-R	2	24.6 ± 0.73	26.4 ± 1.43	21.8 ± 0.53	28.4 ± 0.82
S-T	2	—	—	19.9 ± 0.85	30.0 ± 1.61
U-V	2	—	—	26.5 ± 0.53	25.1 ± 1.16

TABLE 2

Total protein and protein fractions of blood serum

Treatment group and series	N. num.	Overheating series		Overheating of adapted rabbits at 45°	Norm
		Control series	Overheating series		
8	8	3.6 ± 0.18	—	—	3.7 ± 0.15
9	9	0.7 ± 0.06	0.6 ± 0.07	P > 0.5	0.7 ± 0.05
10	10	1.1 ± 0.10	0.7 ± 0.07	P < 0.05	0.9 ± 0.08
11	11	1.6 ± 0.06	1.4 ± 0.31	P < 0.05	1.6 ± 0.10
12	12	7.0 ± 0.11	6.4 ± 0.13	P < 0.05	6.9 ± 0.18
13	13	1.04 ± 0.08	1.36 ± 0.05	P < 0.01	1.15 ± 0.04
14	14	—	—	—	—
15	15	51.2 ± 1.61	57.6 ± 0.87	53.1 ± 1.11	—
16	16	9.9 ± 0.33	9.4 ± 0.95	10.7 ± 0.67	—
17	17	15.6 ± 1.66	11.1 ± 1.0	12.9 ± 1.33	—
18	18	23.3 ± 1.14	21.9 ± 1.79	23.0 ± 1.55	—

The data in Table 3 show that after overheating the change in the protein fractions of blood serum in rabbits is expressed by a decrease in the albumin concentration by 0.9 g % (22.5%), γ -globulin by 0.3 g % (15.8); the total protein dropped by 1.3 g % (18.3%). The protein coefficient did not vary ($P < 0.2$).

These experiments show that acute overheating of experimental animals for 30-60 minutes at 40-45° causes hypoproteinemia and a change in the concentration of the blood serum protein fractions. This phenomenon has been noted by a number of authors [1, 6], who associated hypoproteinemia, hypoalbuminemia, the decrease of the dry residue of the plasma, amount of hemoglobin, erythrocytes, and the decrease of blood viscosity which ensue during the first hour of overheating with the dilution of blood by the fluids entering from the tissues.

Conclusions

1. Overheating of white rats in a heat chamber for 30 minutes at 40° induces hypoproteinemia, hypoalbuminemia, the protein content in the extract of soluble liver proteins drops.
2. Overheating of rabbits in a heat chamber for 60 minutes at 45° leads to a decrease in the total protein of blood serum and to hypoglobulinemia. Hypoproteinemia is more evident upon overheating in a solarium.
3. In unadapted rabbits the shifts in the protein composition of the blood serum upon overheating were more evident than in those adapted.

TABLE 3

Total protein and protein fractions of rabbit blood serum before and after overheating in solarium at 45°

Total protein, protein fractions	Norm		Overheating		
	g*	%	g*	%	
Albumin	4.0 ± 0.14	53.5 ± 1.80	3.1 ± 0.25	P < 0.01	52.0 ± 3.46
α-globulin	0.6 ± 0.06	8.5 ± 0.82	0.5 ± 0.06	P < 0.2	9.1 ± 0.10
β-globulin	0.8 ± 0.04	11.5 ± 0.43	0.6 ± 0.03	P < 0.2	11.0 ± 0.59
γ-globulin	1.0 ± 0.13	21.5 ± 1.73	1.6 ± 0.05	P < 0.05	27.9 ± 3.25
Total protein	7.1 ± 0.16	—	5.8 ± 0.15	P < 0.001	—
A/G	1.3 ± 0.07	—	1.15 ± 0.15	P < 0.2	—

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THE EFFECT OF THYROIDECTOMY ON THE TOLERANCE AND DEGREE OF TISSUE
DEHYDRATION IN ACUTE OVERHEATING

A. A. Nepesov and Ye. P. Serebryakov

A study of the physiology of the thyroid gland is of considerable theoretical and practical interest, especially under conditions of the hot climate of Turkmenia where during a long and dry summer the thyroid function is subjected to the constant effects of the environment.

One of the main climatic factors in a hot climate is the effect of heat. Therefore, in our investigations we studied the effect of heat on the animal organism with the thyroid gland removed.

It is known from the data in the literature [3] that thyroidectomized animals are less tolerant to heat. It was also learned that retention of excess water in an organism occurs in thyroidectomized animals [1]. S. Ya. Kaplanskii [2], having studied the water content in animal tissues and organs, found that upon thyroidectomy the water content in the skin increases whereas in other organs its level remains unchanged.

A disorder of water-salt metabolism upon thyroidectomy of course cannot help but be reflected in physical thermoregulation which, as is known, is accomplished by evaporation of perspiration in man and by

polypnea in most higher animal organisms.

What has been stated induced us to carry out experiments to reveal the role of the thyroid glands during acute hyperthermia.

The survival and degree of dehydration of guinea pig tissues and organs were the indexes of the changes ensuing in the organism during acute hyperthermia. We used 80 guinea pigs divided into two equal groups. We removed the thyroid in the animals of the first group and the animals of the second group served as the control. The postoperative period lasted two weeks, the diet was mixed. Four series of experiments were carried out: ten thyroidectomized and ten control animals were in each series. The animals were subjected to high temperatures in a chamber where they were placed for 5-8 hours. The starting temperature was 35°, it was raised 1° every ten minutes and, having reached 41°, was held there during the experiment.

At the end of the indicated period we counted the number of animals that survived and those that died in both groups. After the death of an animal we determined the degree of tissue and organ dehydration in the guinea pigs of both groups by the dry residue method (Table 1).

TABLE 1

Dehydration of tissues and organs of Guinea pigs with and without thyroid removed in hyperthermia (% of water to weight of organ tissues)

Experimental animals	Heart	Lungs	Liver	Stomach	Brain	Muscles	Skin	Stomach	Small intestine	Large intestine	% of animals
Controls not overheated	79.6	74.25	70.4	79.2	77.57	80.25	77.3	64.76	78.14	74.3	84.61
Overheated controls	79.49	80.71	73.17	78.68	78.41	79.39	76.66	63.62	79.05	79.54	81.92
Not overheated thyroidectomized	79.61	79.45	69.7	78.43	77.81	79.89	76.91	65.9	79.3	79.3	81.5
Overheated thyroidectomized	79.31	81.11	72.49	78.41	78.89	79.4	76.24	57.71	79.41	79.6	82.25

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Results of experiments. Our investigations showed that the degree of survival is not the same in guinea pigs that were thyroidectomized. Of the 40 guinea pigs that were operated on, 34 (85%) died and of the controls, 15 (37.5%). Consequently, the degree of survival of control animals is twice that of the animals with the thyroid removed. These data on the different degrees of survival of the animals are confirmed by experiments which we carried out earlier on white rats by the above-described method. Out of 15 control animals, 12 (80%) survived and out of 15 thyroidectomized animals, only 4 (26%) survived.

The degree of organ and tissue dehydration is also dissimilar in both groups. In animals with the thyroid removed we noted a small increase in the water content of the skin and tissues of the stomach. Upon overheating we noted an increase in the water content of the lungs and liver in both groups. In this set-up of the experiments, dehydration of the animals upon overheating probably occurs by way of the skin, since in animals of both groups the amount of water in the skin decreases relatively more than in other tissues. But this loss in thyroidectomized animals is greater and reaches 8% of the tissue weight, whereas in the control animals it is no more than about 1.7%. Dehydration also takes place in muscles but it is negligible.

The figures reflecting the degree of dehydration of tissues and organs upon overheating of animals permit us to conclude that the mechanism of skin dehydration is disturbed in thyroidectomized animals.

Conclusions

1. Upon acute overheating the degree of survival of thyroidectomized guinea pigs and rats is lower than in normal animals.
2. The water content in skin and tissues of the stomach increases in thyroidectomized animals.

3. Upon overheating the degree of skin dehydration in thyroidectomized animals is higher than those overheated in the control group. In thyroidectomized animals it was about 8% and in the control animals, 1.7%.

4. In overheated animals of both groups we observe an increase in the water content in the liver (in thyroidectomized animals by 2.8% and in the controls by 5.6%).

5. The experimental data can be of definite interest when studying the effect of the hypofunction of the thyroid on the condition of the human and animal organism under conditions of a hot climate.

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THE EFFECT OF OVERHEATING ON SUCCINIC DEHYDROGENASE ACTIVITY IN THE
HEART MUSCLE OF WHITE RATS

E. L. Perel'man

Many investigators are studying overheating. There are a number of works elucidating the shifts of metabolic processes during overheating [1, 2, 3, 4, 7, 8, 9, 10]. But the problem of the change in oxidation-reduction processes which yield the necessary energy to maintain the vital activity of an organism has been elucidated very little.

Succinic dehydrogenase plays an important role in cell respiration. It participates in the transformations of the so-called Kreb's cycle in which the pathways of the intermediate metabolism of carbohydrates and fats are interlinked and simultaneously enters into the succinic oxidative system. The latter performs catalyzing dehydrogenation of succinic acid by the combined action of succinic dehydrogenase, cytochrome oxidase, and cytochrome C [6, 13].

In our investigation we studied the activity of succinic dehydrogenase in the heart muscle of white rats exposed to overheating.

Methods and results of the investigation. We determine the activity of succinic dehydrogenase by the method of tetrazolium salts proposed in recent years [5, 14]. These compounds have the ability to

accept electrons from dehydrogenases and to be stained where succinic dehydrogenase is localized. Tetrazolium salts are water-soluble colorless compounds; on reduction they yield a stained product (formazan) which is not soluble in water.

In our investigations we used triphenyltetrazolium chloride. We introduced certain changes in the method of determining succinic dehydrogenase proposed by E. Kun and L. Abood [12], C. Jardepzky and D. Glick [11].

In the experiments we used 31 white rats (male) weighing 200-250 g. All the investigated animals were on a nutritious mixed diet. Acute overheating was carried out in a hot chamber for 1-1.5 hr. at 42-45°; 14 animals were subjected to overheating and 17 were the control. The animals were decapitated. In order to remove the blood, the heart was perfused through the aorta with 0.01 M phosphate buffer, pH 7.7. Thus we were able to free the blood in the capillaries of the muscle tissue. The remaining blood was washed out of the heart cavity with a buffer solution after incision of the heart muscle; the connective tissue was removed and the muscle dried with filter paper.

Certain authors consider that the presence of blood does not change the activity of succinic dehydrogenase [11]. But the investigations we preliminarily carried out showed that the introduction of blood into the homogenate considerably enhances reduction of tetrazolium. We tried to wash the tissue with water, physiological solution, and phosphate buffer. More distinct shifts were revealed on washing with a buffer solution. Apparently upon contact with the phosphate buffer there occurs a normalization of the colloidal structure which is necessary for the functioning of the succinic dehydrogenase. After washing, the tissue was triturated in a mortar with nine volumes of 0.1 M phosphate buffer, pH 7.7. The homogenate was filtered through one layer of gauze.

We added to the test tubes 0.25 ml of 0.25 M sodium succinate prepared on the basis of 0.1 M phosphate buffer, ph 7.7, 0.25 ml of a 0.4% solution of tetrazolium and 0.5 ml of homogenate. The test tubes were stoppered and incubated in a thermostat for one hour at 38°. The tetrazolium concentration in the experimental test tube was 1 mg/ml (2.89 micromoles) and the concentration of succinate was 0.01 M. The formation of formazan without succinate did not occur in our investigation. A red stain which became intense after one hour, appeared after only 10-15 minutes in the presence of succinate. After incubation the test tubes were centrifuged and the liquid over the residue was removed. We added to the residue 1 ml of glacial acetic acid and placed it in warm water. The residue was ground with a glass rod, afterward we added 5 ml of toluene and briefly shook it. The test tubes were cooled and centrifuged to clear the toluene. Extraction of the formazan in the toluene yielded a stable stain. The optical density of the extracts was measured on a FEK with a green filter (light transmission 490-530 millimicrons). We obtained the calibration curve of formazan (Fig. 1) by reduction of a certain amount of tetrazolium dissolved in the phosphate buffer, ph 7.7, by an excess of hydrosulfite with subsequent extraction by toluene in the presence of glacial acetic acid.

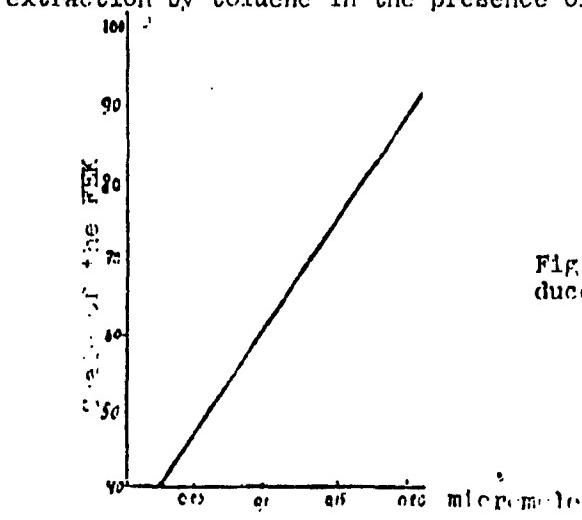


Fig. 1. Calibration curve of reduced tetrazolium.

As we see from Fig. 1 the calibration curve of the reduced tetrazolium shows a linear dependence between the concentration of formazan which was formed and the absorption of light.

In the control animals we determined the activity of succinic dehydrogenase in homogenates of heart muscle, kidneys, liver, and the brain. Our investigation shows that the highest activity was in the heart muscle, than in the liver and the lowest activity was in the brain.

The results of determining the succinic dehydrogenase activity in the heart muscle of 14 overheated and 17 control animals are as follows:

Value of effect	number of animals	Concentration of reduced tetrazolium (micromoles/1 g. of tissue)
Control	17	3.12 ± 0.12
Overheated	14	2.48 ± 0.38 $P < 0.05$

As is apparent from these data, the succinic dehydrogenase activity of the control animals was on the average 3.12 micromoles of reduced tetrazolium; a drop to 2.48 micromoles was noted in the overheated animals. Therefore, under the effect of overheating the succinic dehydrogenase activity decreases in heart muscle.

Previous investigations of the flavoprotein level showed that under the effect of acute overheating the content of flavin-adenine dinucleotides decreases in certain tissues. A comparison of these data with the result of the present investigations show a decrease in the activity of succinic dehydrogenase--an enzyme which also belongs to flavin-adenine dinucleotides--permits us to conclude that in the presence of hyperthermia the activity of single links of the flavin-adenine dinucleotides enzyme group decreases.

Of course the results of determining the enzymes of energy metabolism in homogenates of tissues (stroma, vessels, parenchyma) cannot yield a complete concept of the level of oxidation processes in the given tissue. A study of respiratory enzymes in isolated mitochondria, where the succinic oxidase enzyme system is localized, is the task of our subsequent investigations.

Conclusions

We showed by means of triphenyltetrazolium chloride that under the effects of acute overheating the activity of succinic dehydrogenase decreases in the heart muscle of white rats.

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LEUKOCYTIC FORMULA AND PHAGOCYTIC ACTIVITY OF BLOOD
LEUKOCYTES IN HYPERTHERMIA

A. T. Berdyyeva

A study of the condition of leukocytes enables us to judge the changes in the reactivity of an organism, the character of the course and outcome of an illness, and the effectiveness of therapeutic measures.

Variations in the phagocytic activity of leukocytes are possible in a healthy organism, for example, in relation to the season of the year. In Transbaikalia the greatest activity of leukocytes is noted in the autumn. A decrease in phagocytic properties of the blood has been observed during the winter and spring seasons of the year [3]. From the aspect of the individual leukocytes, it was possible to note in the autumn a definite increase in the absolute number of neutrophil cells [4]. The number of eosinophils and staff leukocytes in monkeys increases during the summer-fall period and the monocytes during the winter-spring period. Not only seasonal, but also daily changes in the number of leukocytes occur in these animals [5].

The antimicrobial function of the reticuloendothelial system decreases in hyperthermia of animals. In guinea pigs subjected to the effect of cold, the administration of a microbial suspension induces

the formation of abscesses, peritonitis, pericarditis, etc. [1, 2].

The leukocytes, consequently, are very sensitive to various types of effects. Therefore, the need to study their changes under very diverse forms of pathological disturbances in an organism is quite evident.

Our task was to study the morphological composition and phagocytic activity of blood leukocytes in animals subjected to the effect of a high temperature of the surrounding medium. The experiments were set up with 34 rabbits which were overheated in a heat chamber and in natural conditions (in a solarium). The blood was collected from the marginal vein of the ear before the experiment and 30 and 60 minutes afterwards.

Several series of experiments were set up during the study. As a control we used the data obtained from rabbits of the control group and from other animals before starting the experiment. Thus, 46 analyses of the blood were processed.

In order to study the phagocytic activity of leukocytes, two volumes of blood were mixed with one volume of a 2% solution of sodium citrate. To this mixture we added one volume of a 1.6 billionth microbial suspension of a daily culture of *Staphylococcus aureus*. After 30-minute incubation in a thermostat at 37° we made a smear which was then fixed with ethyl alcohol. Staining was done by Giemsa-Romanowsky's method. The blood smears intended for studying the leukocytic formula (leukogram) were processed in the same manner. The data of the relative variation in the total number of leukocytes in the blood were obtained in the same animal. The following data were obtained in the control. The total number of leukocytes was 12,458, of which 0.14% pertained to myeloblasts, 1.21% to myelocytes, 9.05% to young, 12% to staff, and 40.36 to segmented leukocytes. In the total complexity 7811

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fell to the lot of neutrophil cells, which was 62.7% of the total number of leukocytes. This index is important since neutrophils participate mainly in the phagocytic reactions of an organism. The portion of eosinophils was 2.07%, basophils 1.16%, monocytes 3.65%, and lymphocytes 30.45% (Table 1).

In the control we determined the average figures of the phagocyte number (the number of leukocytes participating in phagocytosis) and the phagocyte index (the number of microbial bodies absorbed on the average by each phagocyte). These figures were respectively equal to 55.52% and 1.81.

In our experiments the rabbits were subjected to overheating in a hot chamber at 45°. In the first series of experiments the animals underwent preliminary daily adaptation for one hour over a period of 14 days. The second group of animals was used in the experiment without such adaptation. In both cases there was an appreciable drop in the total number of leukocytes, which was especially evident in the second group of rabbits (to 5025 per 1 mm³). Upon overheating without adaptation we observed after 30 minutes a significant increase in the phagocyte number (72%) and the phagocyte index (to 2.11). By the end of the experiment, that is after 60 minutes, the phagocyte number drops (65.53%) and the phagocyte index continues to grow (to 2.22). This fact is interesting because at this period of time a decrease in the number of leukocytes by almost half was observed in all animals. Therefore, we can assume that the organism, as it were, endeavors to increase the phagocyte indexes in order to compensate for the lack of leukocytes in the blood. In adapted animals these indexes, which were determined at the beginning and the end of the experiment, differ very little from one another (60-60.7%; 2.12-2.1%). However, in the middle of the experiment we again noticed an increase in the phagocyte number (67.4%).

The total number of leukocytes (to 9600) in these animals did not drop severely. We get the impression that adaptation to high temperatures, so to speak, fosters adaptation of an organism to new, unusual environments. The organism as usual strives to retain a relatively constant quantitative and qualitative blood composition.

In regard to the morphological composition of the blood, we must note that overheating without adaptation is accompanied by a decrease in the per cent of neutrophil cells, which is especially noted after 30 minutes (73.7; 67.23; 70.08%). The number of mature, segmented leukocytes decreases most of all (45; 42.72; 34.61%). The content of myelocytes increased to 2.88%.

In the adapted rabbits the number of neutrophils did not vary so noticeably (67.16; 66.10; 70.93%). The number of segmented cells at first increases and then returns to the norm, and the number of segmented and juvenile leukocytes is normalized by the end of the experiment. The number of monocytes increases considerably (2.53; 3.6; 4.64%).

It is interesting to note that on the sixth day of adaptation with the animals in the heat chamber, very young cells, myeloblasts (0.12%) and lymphoblasts (0.24%) appear in the blood after the experiment. The number of eosinophils (0.87; 2.5%) and monocytes (2.25; 6.53%) increases appreciably.

It is necessary to state that an increase in the number of myelocytes was noted in all cases of overheating the animals; moreover, we frequently noted phagocytosis in the eosinophils. Overheating of the animals in the solarium was carried out against a background of pre-induced hemorrhage. The combination of these two factors induced a drop in the total number of leukocytes (to 8700 per 1 mm³), the morphological composition changed negligibly. The phagocytic number decreased sharply (55.82; 40.81%). The phagocytic index also somewhat decreased

(1.81; 1.08). In all probability this fact can be explained by the effect, not so much of overheating, as by the loss of blood.

According to the data of V. S. Tarasova [6], blood transfusion is a powerful factor for stimulating the phagocytic capacity of leukocytes. Transfusion of blood induces even greater shifts of the opsono-phagocytic index than nonspecific immunization. In our experiments accompanied by considerable hemorrhage (up to 20%), we noted a drop in the phagocytic function of leukocytes which increases under the effect of a single overheating. It is possible that hemorrhage causes some kind of disorders in the regulation of the leukocytic reactions of an organism to the effect of high temperature. This problem should be refined by special investigations with overheating under natural conditions without the influence of additional factors.

Conclusions

1. Upon overheating of animals, an increase in the phagocytic indexes of the blood is observed simultaneously with a decrease in the total number of leukocytes.
2. The change in the phagocytic number and phagocytic index of leukocytes is especially evident in animals not adapted to high temperature.
3. During adaptation, the appearance of myeloblasts, lymphoblasts, as well as monocytosis and eosinophilia is observed in the blood.
4. Overheating of adapted animals is accompanied by neutrophilia and overheating of unadapted animals by neutropenia.
5. A combination of overheating in a solarium with hemorrhage induces a drop in the phagocytic indexes of the blood.

TABLE I
Change of rabbit Blood Formula on Overheating

Results Taken	Leukocytes										total lymphocytes
	melo- blast	myelo- blast	juve- nile	staff	seg- mented	eosin-	mono-	lympho-	lympho-		
control	0.11 1.14	1.21 1.34	0.65 1.45	12.00 11.95	40.30 30.05	2.07 25.58	1.16 14.51	3.65 45.73	0 0	30.42 37.97	12458
5 hr. day 3° overheating	0 0	0.38 17.54	5.75 71.84	9.25 115.23	40.50 50.645	0.85 16.335	0.12 1.95	2.25 28.131	0.12 14.93	10.75 30.7691	12458
5 hr. day 3° overheating	0.12 1.67	0.63 3.91	3.75 37.591	8.62 97.16	39.61 391.90	2.50 250.62	0 0	6.53 65.163	0.24 24.06	38.00 330.950	10025
Overheating at +5° after adaptation	0 0	0.51 6.34	1.30 134.79	17.69 21.32	26.56 45.5165	2.31 257.73	0.20 2.60	2.53 31.18	0 3.60	27.80 3463.32	12458
Overheating at +5° after adaptation	0 0	0.29 22.14	0.70 105.80	20.00 21.1410	46.20 510.94	0.20 51.31	0.20 22.14	0.20 39.52	0 0	17.50 1936.23	11070
Overheating at +5° after adaptation	0 0	1.13 17.42	13.35 27.17	16.90 21.46	39.34 37.4135	2.42 232.56	0.20 19.22	4.64 45.98	0 4.51	21.81 2195.92	9610

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Continuation of TABLE 1

Period taken:	Leukocytes						total lymphocytes
	mixed-melano- blast	myelo- cyte	true- nile	seg- mented	eosin- ophil	mono- phl	
Overheating at -5° without adaptation							
3. exp. overheating	0	1.20	14.50	12.40	45.30	2.30	1.10
	0	149.50	1513.70	1514.70	5643.45	224.25	386.19
1. exp. 30 min.	0	0	13.20	11.20	42.72	3.76	1.14
	0	0	32.00	24.72	247.72	2.47	0.72
2. exp. 60 min.	0	2.28	19.14	12.68	34.61	2.23	1.41
	0	114.72	1601.48	1735.56	1735.56	112.65	71.85
Overheating in the solarium							
1. exp. overheating	0	1.49	4.50	1.60	5.50	2.00	0.27
	0	167.13	697.19	715.67	4765.12	291.16	333.61
2. exp. 30 min.	0	1.32	5.79	18.79	42.61	1.54	0.69
	0	153.71	57.85	739.21	3433.3	131.36	7.55

NOTE: In the numeration is the per cent of individual forms of leukocytes in 1 mm³; in the denominator is their absolute number.

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THE EFFECT OF OVERHEATING ON THE RIBOFLAVIN IN WHITE RAT TISSUES

E. L. Perel'man

Numerous investigations have established that substantial changes in the vitamin level occur in animals under high-temperature conditions. The studies of a number of authors showed that almost all known vitamins are present in the sweat composition [3, 4, 6, 7, 9]. The experiments of other investigators on animals, for which thermoregulation by means of sweating was not important (rats, chicks), showed an increased vitamin requirement upon overheating [9, 10]. These data show that the increase vitamin requirement under high-temperature condition is probably associated with the change in the rate of metabolic processes [1, 4]. But there are very few investigations in this direction.

It is of interest to study the role of riboflavin in metabolism under the effects of high temperatures. Riboflavin fulfills various functions in an organism which are directly associated with processes of growth and nutrition [2, 4]. It has now been established that riboflavin (ORF) and its compounds flavin mononucleotide (FMN) and flavin-adenine dinucleotide (FAD) enter into the enzymatic system of flavoproteins and participate in biological oxidation. During cellular respiration the hydrogen of the oxidized substrate is taken up by dehydrogenases

and then is transferred to flavoproteins. The flavoproteins transfer the hydrogen to a system of cytochromes or to oxygen with the formation of the end product of oxidation [5].

In the present work we investigated the effect of overheating on the content of total riboflavin, flavin mononucleotide and flavin-adenine dinucleotide in white rat tissues. The experiments were set up with 42 white rats (males) weighing 200-250 g, which were divided into four groups. The rats of the first group underwent acute overheating in a special chamber for 1-1.5 hours at 42-45°. In this case the rectal temperature of the rats increased to 42-42.5°. In order to adapt the animals to heat, those of the second group were heated in a chamber at 35-36° for one hour daily for a month; the third group of animals consisted of adapted rats, which underwent acute overheating. The fourth group was the control. The diet for all animals consisted of groats, vegetables, casein, and grasses. The animals were killed by decapitation. A fluorometric measurement of ORF, FMN, and FAD was carried out by Bessey's method [8] in the extracted organs. We did not determine the amount of free riboflavin since its content was small and determination is complex, having conditionally considered that ORF minus FAD is FMN. Extraction of ORF was done in the cold by triturating a weighed samples of tissues with glass sand in a cool vessel with 10-20 volumes of water. Then the protein was precipitated by adding an equal volume of 20% trichloroacetic acid. After ten minutes the samples upon cooling were centrifuged. To the transparent centrifugate we added a $\frac{1}{4}$ volume of 4 M K_2HPO_4 . The remaining portion of the centrifugate was left for hydrolysis overnight in a thermontat at 28°. The test tubes with the samples were stoppered with paraffin corks. After hydrolysis the samples were also neutralized with 4 M K_2HPO_4 . To avoid losses of FAD (owing to enzymatic action) the tissues were prepared

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in red light. The degree of fluorescence was determined on the EF-3 fluorometer. Three readings were taken during fluorometry; the initial (F_1), after the addition of a determined amount of riboflavin standard (F_2), and after the extinction of fluorescence by the addition of a $\frac{1}{100}$ volume of 10% $\text{Na}_2\text{S}_2\text{O}_4$ prepared on the basis of 5% NaHCO_3 (F_3). The readings of F_2 and F_3 were corrected for the dilution of the added standard and reducing agent. The content of ORF was calculated by the formula $\frac{F_2 - F_3}{F_2 - F_1}$.

The obtained data of the content of ORF, FAD, and FMN in the tissues of the control white rats corresponds in the main to Lowry's data [6]. The distribution of ORF with respect to FAD and FMN in the individual organs was different, but for each organ this relationship was more or less constant.

The results of our investigations are shown in Table 1.

TABLE 1
Riboflavin Content in White Rat Tissue (micrograms
per 1 g of fresh tissue weight)

Character of action	Number of animals	F_{ORF}	F_{FAD}	F_{FMN}	F_{ORF}	F_{FAD}	F_{FMN}	F_{ORF}	F_{FAD}	F_{FMN}	F_{ORF}
Central	12	2.43 (77.0)	18.03 (0.61)	27.30 (59.9)	20.49 (83.5)	2.99 (75.9)	2.94 (91.5)	2.29 (92.6)	0.14 (0.16)	0.10 (0.12)	
Acute overheating	11	2.43 (75.7)	17.85 (0.59)	29.41 (66.0)	22.76 (60.4)	3.26 (65.0)	2.66 (84.0)	2.32 (91.8)	0.08 (0.12)	0.32 (0.12)	
Heat-adapted	10	2.87 (73.5)	19.21 (0.19)	29.16 (71.0)	25.75 (83.5)	3.31 (76.9)	3.07 (78.0)	2.78 (82.8)	0.16 (0.16)	0.37 (0.18)	
Acute overheating of heat-adapted	9	2.45 (73.0)	19.01 (0.19)	29.40 (69.5)	28.15 (61.0)	3.35 (61.5)	3.41 (80.0)	3.04 (83.4)	0.12 (0.12)	0.19 (0.19)	

NOTE: Data of the FAD Content in % of ORF are shown in parentheses.

The data of Table 1 show that under the effect of prolonged exposure to moderate heat a certain increase in the content of total riboflavin was observed in all investigated tissues. This increase reached

the highest values in tissues of the brain, liver, and skeletal muscle where it was 18-25% of the ORF content of the control. We also observed a change in the relative content of FAD and FMN: the FAD content increased in the kidneys and noticeably decreased in the lungs and skeletal muscles. Correspondingly, the FMN content decreased in the kidneys, in lung tissue, and in the skeletal muscle. Upon acute overheating of the control and adapted animals, the FAD content decreased in the kidneys and spleen and the FMN content correspondingly increased.

Therefore, our investigations showed that the content of flavoproteins changes under the effect of overheating. The activity of the flavin mononucleotide dehydrogenase in a number of tissues increased on overheating, and flavin-adenine dinucleotides decreased. These shifts can indicate a change in the status of energy exchange in white rat tissues upon overheating.

Conclusions

1. The content of riboflavin in tissues increased somewhat upon prolonged exposure to moderate heat. The content of flavin-adenine dinucleotides increased in the kidneys, lungs, and skeletal muscle, and the content of flavin mononucleotides respectively decreased.
2. Upon acute overheating the activity of flavin mononucleotides increased in the kidneys and spleen and the activity of flavin-adenine dinucleotides decreased.

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